HDL-CHOLESTEROL Direct Method

Reagent for quantitative determination of HDL-cholesterol in human serum or plasma

**IN VITRO DIAGNOSTIC USE**

**TECHNICAL SUPPORT AND ORDERS**
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**CLINICAL SIGNIFICANCE**

The principal role of high density lipoproteins (HDL) in lipid metabolism is the uptake and transport of cholesterol from peripheral tissues to the liver through a process known as reverse cholesterol transport. Low HDL cholesterol levels are strongly associated with an increased risk of coronary heart disease and coronary artery disease. Hence, the determination of serum HDL-Cholesterol is a useful tool in identifying risk of atherosclerosis.

**PRINCIPLE**

Accelerator selective detergent methodology.

Direct method, without specimen pre-treatment.

During the first phase, LDL, VLDL particles and Chylomicrons generate free cholesterol, which through an enzymatic reaction, produce hydrogen peroxide. The generated peroxide is consumed by a peroxidase reaction with DSBmT yielding a colourless product.

During the second phase, specific detergent solubilises HDL-Cholesterol. In conjunction with Co and Ce action, POD + 4-AAP develop a coloured reaction which is proportional to HDL-Cholesterol concentration. The absorbance is measured at 600 nm.

**REAGENTS COMPOSITION**

**Vial R1**

**ACCELERATOR**

<table>
<thead>
<tr>
<th>Good's Buffer</th>
<th>CO</th>
<th>POD</th>
<th>DSBmT</th>
<th>Accelerator</th>
<th>Preservative</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 1000 UI/L</td>
<td>&lt; 1300 ppb UI/L</td>
<td>&lt; 1 mmol/L</td>
<td>&lt; 1 mmol/L</td>
<td>&lt; 0.06 %</td>
<td></td>
</tr>
</tbody>
</table>

**Vial R2**

**SELECTIVE DETERGENT**

<table>
<thead>
<tr>
<th>Good's Buffer</th>
<th>CE</th>
<th>4-AAP</th>
<th>Detergent</th>
<th>Stabiliser</th>
<th>AAO</th>
<th>Preservative</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 1500 UI/L</td>
<td>&lt; 1 mmol/L</td>
<td>&lt; 1 mmol/L</td>
<td>&lt; 2 %</td>
<td>&lt; 0.15 %</td>
<td>&lt; 3000 UI/L</td>
<td>&lt; 0.06 %</td>
</tr>
</tbody>
</table>

**REF 95506 BIOLABO HDL / LDL / CK MB CALIBRATOR**

Vial R1 (lyophilisate): 1 x 2 mL  Vial R2 (diluent): 1 x 5 mL

See enclosed Batch specific Package Insert

**REAGENTS PREPARATION**

Reagents are ready for use.

**STABILITY AND STORAGE**

Store at 2-8°C well recap in the original vial and away from light

- When used and stored as described in the insert, unopened reagents and calibrator are stable until expiry date stated on the label.
- Once opened, when free from contamination, reagents R1 and R2 are stable at least for 3 months at 2-8°C. 24 h at room temperature and 30 days on board in refrigerated analysers.
- Once reconstituted, REF 95506: refer to package insert (batch specific)

**SAFETY CAUTIONS**

BIOLABO reagents are designated for professional, in vitro diagnostic use.
- Verify the integrity of the contents before use.
- Use adequate protections (overall, gloves, glasses).
- Do not pipette by mouth.
- In case of contact with skin and eyes, thoroughly wash affected areas with plenty of water.
- Reagents contain sodium azide (concentration < 0.1 %) which may react with copper and lead plumbing. Flush with plenty of water when disposing.
- For further information, Material Safety Data Sheet is available upon request.
- Waste disposal: Respect legislation in force in the country.

**SPECIMEN COLLECTION AND HANDLING**

Specimens should be handled as potentially infectious, in accordance with good laboratory practices using appropriate precautions. Respect legislation in force in the country.

**INTERFERENCES**

Tested concentrations (mg/dL) without significant interference (±10%):
- Direct bilirubin: 60
- Total bilirubin: 60
- Hemoglobin: 1000
- Ascorbic acid: 100
- Lipemia (Intralipid®): 1800
- Endogenous triglycerides: 2000
- Gamma-globulins: 5000

This reagent may interfere with the magnesium determination.

**SAFETY PRECAUTIONS**

Manufacturer Use by In vitro diagnostic Temperature limitation Catalogue number See insert Batch number Store away from light sufficient for dilute with

Made in France Latest revision: www.biolabo.fr Revision: 15/12/2011

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MATERIAL REQUIRED BUT NOT PROVIDED
1. Basic medical analysis laboratory equipment
2. BIOLABO HDL-Cholesterol calibrator REF 95406 or any traceable calibrator of human origin.
3. HDL LDL CK-MB Calibrator REF 95506
4. or any traceable calibrator (human origin).
5. HDL LDL CK-MB controls (human origin) REF 95516 HDL LDL CK-MB Control Level 1 REF 95526 HDL LDL CK-MB Control Level 2
6. or any normal and pathological control sera of human origin.

QUALITY CONTROL
• Do not use aqueous calibrator
• Use BIOLABO HDL-Cholesterol Calibrator REF 95406 traceable to CDC reference method (secondary standard HDL-M04).
• Or HDL LDL CK-MB Calibrator REF 95506 traceable to SRM® 1951b (Standard Reference Material®) assayed on CDC (Center for Disease Control)
• Or a calibrator of human origin traceable to reference method or material.

The calibration frequency depends on proper instrument functions and on the preservation of the reagent.

It is recommended to calibrate in the following cases:
1. When changing vial of reagent.
2. After maintenance operations on the instrument.
3. When control values obtained are out of range, even after using a new vial of fresh serum.
4. If control is still out of range, calibrate with a new vial of fresh serum.
5. If control is still out of range, please contact BIOLABO technical support.

QUALITY CONTROL
• REF 95516 HDL LDL CK-MB Control level 1
• REF 95526 HDL LDL CK-MB Control level 2
• Or any assayed control sera of human origin referring to the same method (Selective detergent).

EXPECTED VALUES
<table>
<thead>
<tr>
<th>HDL-Cholesterol</th>
<th>mg/dL</th>
<th>[mmol/L]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low level (Risk factor)</td>
<td>&lt; 40</td>
<td>&lt; 1.0</td>
</tr>
<tr>
<td>High level (Protective factor)</td>
<td>&gt; 60</td>
<td>&gt; 1.5</td>
</tr>
</tbody>
</table>

Each laboratory should establish its own normal ranges for the population that it serves.

EXPECTED VALUES (6)

PERFORMANCES CHARACTERISTICS (4)

LINEARITY
The reaction is linear from 2.5 to 200 mg/dL (0.065 to 5.17 mmol/L). Above, dilute the specimen (1+1) with saline solution and reassay applying dilution factor 2 to calculate the result. Linearity limit depends on specimen/reagent ratio.

MANUAL PROCEDURE
Do not use aqueous calibrator
Let stand reagents and specimens at room temperature.

Set up the instrument to read micro-volumes.

<table>
<thead>
<tr>
<th>Reagent R1</th>
<th>Calibrator</th>
<th>Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>300 µL</td>
<td>300 µL</td>
<td>300 µL</td>
</tr>
<tr>
<td>3 µL</td>
<td>3 µL</td>
<td>3 µL</td>
</tr>
</tbody>
</table>

Specimen

Mixer vigorously, let stand for 5 minutes at 37°C.
Record absorbance A1 at 600 nm against reagent blank

Add

<table>
<thead>
<tr>
<th>Blank</th>
<th>Calibrator</th>
<th>Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 µL</td>
<td>100 µL</td>
<td>100 µL</td>
</tr>
</tbody>
</table>

Mixer vigorously, let stand for 5 minutes at 37°C.
Record absorbance A2 at 600 nm against reagent blank

Notes:
1. Depending on the instrument specifications, one can modify the above volumes taking into account the same dilution ratio (i.e. R1 240 µL, R2 80 µL, specimen 2.4 µL or 3 µL). Refer to § LINEARITY.
2. Specific procedures are available upon request for automated instruments. Please contact BIOLABO technical support.

CALCULATION
Automatic analyser with bichromatic reading (600 - 700 nm), is recommended.

With manual procedure, calculate \( \Delta \text{Abs.} = (A2 - 0.75 \text{ A1}) \) for assay and calibrator.

Calculate the result as follows:

\[
\text{HDL-C} = \frac{\Delta \text{Abs. \ Assay}}{\Delta \text{Abs. \ Calibrator}} \times \text{Calibrator concentration}
\]

mg/dL x 0.02586 = mmol/L

REFERENCES
(3) Gotto, A.M., Lipoprotein metabolism and the ethiology of hyperlipidemia, Hospital Practice, 23, Suppl. 1, 4 (1988)

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